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Late Postembryonic Development of the Symbiotic Light Organ of *Euprymna scolopes* (Cephalopoda: Sepiolidae)

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Abstract. The symbiotic light organ of the sepiolid squid *Euprymna scolopes* undergoes significant anatomical, morphological, and biochemical changes during development. Previously we described the embryonic organogenesis and early postembryonic development of the light organ. During embryogenesis, tissues are developed that will promote the onset of an association with *Vibrio fischeri*, the light organ symbiont. Upon inoculation, and in response to the first interactions with the bacterial symbionts, the light organ undergoes a dramatic morphogenesis during the first 4–5 days of postembryonic development. Here we describe the final developmental stage of the light organ system, a period of late postembryonic development in which particular tissues of the light organ mature that eventually mediate the functional symbiosis. The maturation of the light organ occurs within 1 to 2 weeks posthatch and entails two principal processes: (1) changes in the shape of the organ and elaboration of the accessory tissues that modify the bacterially produced light; and (2) branching of the epithelial crypts, where the bacterial symbionts reside, and restriction of epithelial cell proliferation to the deepest branches of the crypts. The gross morphological changes of the organ occur in the absence of *V. fischeri*, although rudiments of the ciliated field of the hatchling remain in animals not exposed to the microbial symbiont.

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Introduction

A variety of mutualistic symbioses exist between bacteria and many species of plants and animals. The associations can be highly evolved and complex, involving the development of specific host tissues or organs that directly interact with the bacterial symbionts; examples include the root nodule of leguminous plants (Hirsch, 1992), the rumen of ungulate mammals (Flint, 1997), the light organs of some species of squid and fish (Herring, 1978; Haygood, 1993), the trophosome of vestimentiferans (Jones and Gardiner, 1988), and the bacteriocytes of certain insects (Douglas, 1989). Several of these associations have "indirect" transmission of symbionts between generations; that is, onset of the symbiosis results from interactions with environmental microbes, not microbes that are in or on the eggs.

The early morphology of these organs, prior to the infection with symbionts, is often quite different from that of the fully differentiated symbiotic state (Buchner, 1965; Jones and Gardiner, 1988; Fisher and Long, 1992; Hirsch, 1992; Montgomery and McFall-Ngai, 1993, 1994). In the well-characterized *Rhizobium*–leguminous plant symbioses, stages of the symbiosis have been defined as preinfection, infection, colonization, and maturation (van Rhijn and Vanderleyden, 1995). These stages correspond to specific molecular, biochemical, and anatomical changes in both the host plant and the bacterial symbiont that reflect the progression of the association from initiation to the mature nitrogen-fixing symbiosis. More than 100 years of research on the host plants and bacterial symbionts have revealed that dozens of host and symbiont genes are induced in the progression of these developmental changes.

The symbiosis between the sepiolid squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri* provides an opportunity to define the events associated with the development of an *animal* symbiosis (McFall-Ngai and Ruby, 1991, 1998; Ruby, 1996). The development of the light organ can be divided into 3 distinct stages—embryonic, early postembryonic, and late postembryonic—reflecting the preinfection, inoculation, and maturation stages of the symbiosis. During embryonic development, an incipient organ that is primarily structured to ensure inoculation with the bacterial symbionts forms in the mantle cavity. This organ includes a complex, superficial, ciliated epithelium that will potentiate inoculation as well as a set of epithelium-lined crypts, or inpocketings of the surface of the light organ, that will house the symbiotic bacteria (Montgomery and McFall-Ngai, 1993). Early postembryonic development is induced by initiation of the symbiosis and is characterized by the cell-death-mediated loss of the ciliated epithelium and the further differentiation of the crypt cells that directly interact with the symbionts (Montgomery and McFall-Ngai, 1994; Lamarcq and McFall-Ngai, 1998). In the mature organ, the luminescent bacterial symbionts are maintained in a complex bilobed light organ. The functional symbiotic organ is composed of several host tissues, including a core of branching epithelial crypts that houses the symbionts; a thick reflector that directs the bacterial luminescence ventrally; diverticula of the ink sac that dynamically control the intensity of luminescence; and a ventral muscle-derived lens that diffuses the point-source light into the environment (McFall-Ngai and Montgomery, 1990). In addition, two bright yellow filters, which are situated ventral to the bacteria-containing tissue and dorsal to the lens, are associated with the ducts of the adult organ (pers. obs.; Fig. 1). The configuration of adult tissues appears to maintain a stable symbiotic association and to control luminescence, which is apparently used in antipredatory behaviors (Moynihan, 1983).

We have previously described the embryonic and early postembryonic stages of light organ development (Montgomery and McFall-Ngai, 1993, 1994). Here, we provide a biochemical, cytological, and morphological analysis of the changes that accompany the transition from the early postembryonic stages to the mature, functional light organ symbiosis.

Materials and Methods

Light microscopic observations of the light organs of live and fixed specimens

Specimens of *Euprymna scolopes* were collected from Hawaii, as previously described (Weis *et al.*, 1993). Field-caught animals at various developmental stages (ranging in mantle length from 2 to 20 mm) were anesthetized by having their body temperature lowered to 10°C. The ventral side of the mantle and then the funnel were dissected open to expose the light organ within the mantle cavity. Light organs were photographed with a Nikon camera attached to a Wild M-5 stereomicroscope.

Light organs were fixed for light microscopy as previously described (Montgomery and McFall-Ngai, 1993). Serial 1-μm sections of the organs were reconstructed to reveal morphological features.

BrdU incorporation and visualization

To locate active areas of host cell proliferation, field-caught juveniles ranging in mantle length from 6 to 12 mm were exposed for various times, from 12 h to 5 d, to 10 μM 5-bromo-2′-deoxyuridine (BrdU), a thymidine analog that was added to filtered seawater. Animals were transferred to filtered seawater for 30 min and then fixed and embedded as described by Montgomery and McFall-Ngai (1994). One-micron sections were dried on gelatin-coated slides. To denature the DNA, sections were treated with 4 N HCl for 10 min, followed by a neutralizing step in 0.1 M sodium borate (Migheli *et al.*, 1991). Sections were incubated for 30 min in blocking solution consisting of a 1:50 dilution of non-immune goat serum in 10 mM sodium phosphate buffer, containing 150 mM NaCl, 0.05% sodium azide, and 0.5% bovine serum albumin (PBS/BSA). The sections were then rinsed in PBS/BSA and incubated overnight in a 1:50 dilution of primary antiserum, *i.e.*, monoclonal anti-BrdU antibody (Becton-Dickinson, CA); the bound antibody was visualized by light microscopy using goat anti-mouse secondary antibody conjugated to 5-nm gold spheres amplified by silver enhancement (Sigma Chemical Co.). The sections were counterstained with 1% acid fuchsin for 20 min, rinsed in water, dried, and mounted in heavy immersion oil.

Immunocytochemistry

Light organs of field-caught *E. scolopes* were fixed and processed for immunocytochemistry as described in Weis *et al.* (1993). Serial sections, 1 to 1.5 μm thick, were cut with glass knives and dried onto gelatin-coated glass slides. L-crystallin, a light organ lens marker protein, was localized in the tissue sections with anti-L-crystallin rabbit antiserum (Montgomery and McFall-Ngai, 1992; Weis *et al.*, 1993); the antiserum has been shown in previous experiments to be highly specific to this antigen. Bound antibody was visualized in light microscopy with a goat anti-rabbit secondary antibody conjugated to 5-nm gold spheres amplified by silver enhancement (Sigma Chemical Co.). The sections were counterstained with 1% acid fuchsin as described above.

Experiments with aposymbiotic animals

Juvenile animals were maintained aposymbiotically; *i.e.*, they were raised in artificial seawater for 2–3 weeks posthatch at the University of Texas Medical Branch (Galveston, TX). Whole animals were fixed in Texas in 4% formalin in seawater and transported to the University of Hawaii, where they were processed for scanning electron microscopy as previously described (Montgomery and McFall-Ngai, 1994).

Results

Changes in gross morphology of the light organ

Changes in overall gross morphology accompany postembryonic development of the light organ (Fig. 1). A newly hatched juvenile with an average mantle length of 1.6 mm possesses an incipient light organ located in the center of the ventral surface of the ink sac (Fig. 1A; Montgomery and McFall-Ngai, 1993). This juvenile organ is ovoid and lacks a lens, although the silver reflector that covers the ventral surface of the ink sac is apparent. During the first several days of posthatch development, the anterior and posterior diverticula of the ink sac expand and contract to cover the bacteria-containing crypts of the organ (Fig. 2, top).

By the time a juvenile reaches 2.8 mm in mantle length, the light organ has begun to acquire a more bilobed appearance (Fig. 1B). A medial portion of the ink sac, extending from the posterior end, covers much of the center ventral surface of the organ, and diverticula of the ink sac have moved in from the lateral margins, covering the lateral central portion of each lobe. Most of the growth in each lobe has been directed anteriorly, although some posterior growth has also occurred. In each lobe, the lens, although not very apparent, has begun to differentiate. In addition, a pair of yellow filters has begun to form lateroventral to the bacteria-containing crypt tissue.

In juveniles 4.0 mm in mantle length (Fig. 1C), all the components of the light organ of an adult squid are fully differentiated. The characteristic bilobed shape of the adult light organ is evident, and the medial portion of the ink sac that covers the center of the ventral surface of the organ has begun to expand laterally. The bacteria-containing crypts are now covered by a series of dynamic diverticula that wrap around the organ; these diverticula expand and contract, over a period of seconds to minutes, in the anterior-posterior direction and the medial-lateral direction (Fig. 2, middle). In addition, the yellow filters, which are very conspicuous by this stage, are observed in various positions along the anterior-posterior axis, spreading over the bacteria-containing crypt tissue (Fig. 1B–D).

Continued growth of the light organ beyond this stage results in a structure only slightly more well defined and complexly shaped (Fig. 1D). The lateral "flaps" of the ventral medial portion of the ink sac have expanded so that the central half of each lobe is covered. Laterally, each lobe has grown anteriorly and posteriorly, with the lens forming a thick pad over each end and thinning in the middle near the pore; the medial portion of the organ, however, has been almost completely overlain by the posterior ventral fold of the ink sac. The medial and lateral diverticula of the ink sac have become the primary mechanism by which the bacteria-containing tissue of the organ is shielded (Fig. 2, bottom).

Late postembryonic development of the crypts and ciliated duct

Serial histological sections of the light organs of two squids, one 2 mm and the other 8 mm in mantle length, revealed that, although they are highly branched, there are always only three bacteria-containing crypts in a mature light organ (Fig. 3). Each branching epithelial crypt joins the ciliated duct separately; the crypts were identified on the basis of their relative positions to each other and the sites at which each joins the duct. At hatching, the three crypts empty into three separate ducts that lead to three separate pores on the surface of the light organ (Montgomery and McFall-Ngai, 1993). By 2 mm in mantle length, the three crypts on each side of the light organ are joined to a single tripartite duct leading to a single pore (data not shown). In the fully differentiated light organ of the squid 8 mm in mantle length, the ciliated duct, rather than being tripartite, branches into five parts (Fig. 3); *i.e.*, in addition to branches leading to the three crypts, an anterior portion of the duct leads into the inner core of the anterior yellow filter, and a posterior portion leads into the inner core of the posterior filter.

Proliferating cells could be localized by analyzing the incorporation of the thymidine analog BrdU into replicating DNA. Regional differences were observed in the distribution of dividing cells in the light organ of a squid 8 mm in mantle length (Fig. 4A). Only a few cells were labeled in such accessory tissues as the lens (Fig. 4A, B). Cells in the distal portions of the crypts incorporated BrdU intensely (Fig. 4A, C), whereas none of the cells in either the ciliated duct or portions of the crypts close to the ciliated duct was labeled. The morphology of the cells in the proliferating zones of the crypts appeared different from those nonproliferating cells located closer to the duct. The epithelial cells of the distal portions of the crypts are roughly cuboidal and relatively small (Fig. 4C); as cells are located closer to the ciliated duct, they

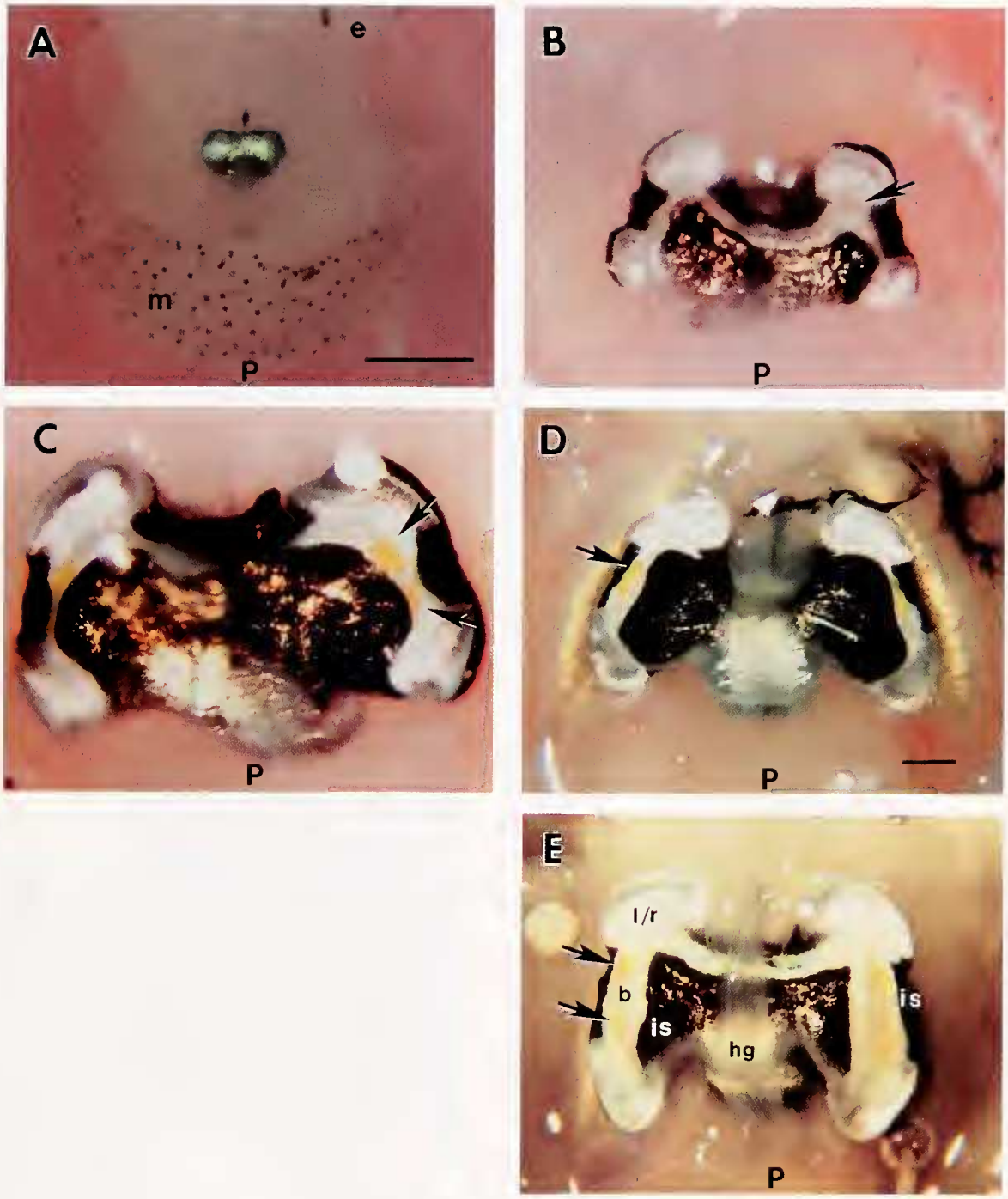

Figure 1. Postembryonic development of the light organ of *Euprymna scolopes*. [Animals were placed against a pink background to provide contrast to the squid light organ tissues.] Ventral views of the light organ of a (A) newly hatched juvenile 1.6 mm in mantle length; m, mantle; e, eye; (B) juvenile 2.8 mm in mantle length; (C) juvenile 4.0 mm in mantle length; (D) sexually mature male 18 mm, and (E) 20 mm in mantle length. The bacteria-containing tissue (b) is dorsal to the yellow filters (arrows in B–D) and appears opaque against the brilliantly silver reflector. The reflector and the lens (l/r), which occurs ventral to the reflector, run much of the length of each lobe. Diverticula of the ink sac (is) expand and contract over the bacteria-containing tissue to control the intensity of light emission. Bar (A–C), 500 μm, (D–E), 1 mm; hg, hindgut; P, posterior.

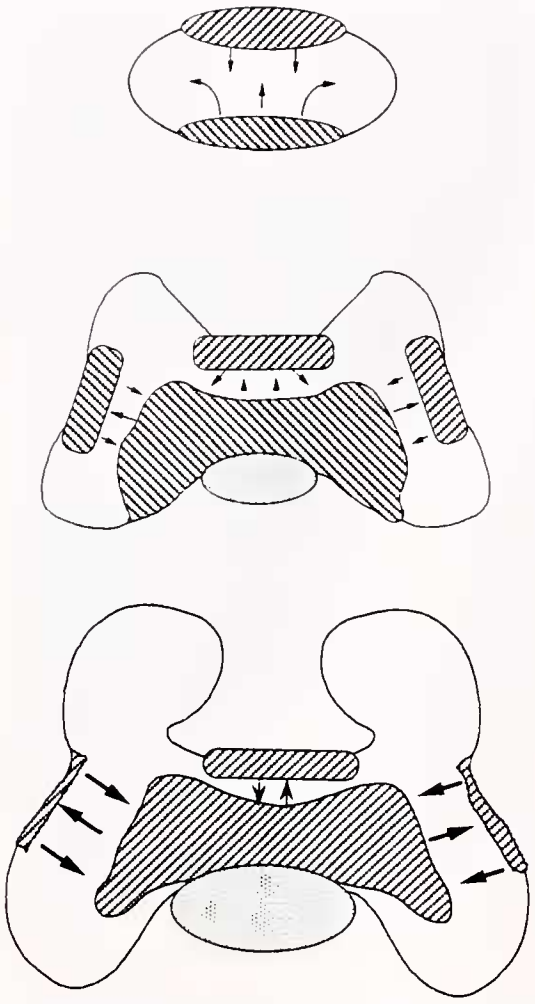

Figure 2. Change in the dynamics of the ink sac diverticula with development of the *Euprymna scolopes* light organ. Top panel, organ of a hatchling (1.6 mm in mantle length); middle panel, juvenile organ (4.0 mm in mantle length); bottom panel, organ of mature adult (20 mm in mantle length). The organs are in the fully "open" position; the arrows indicate the direction of movement of the ink sac diverticula when they expand for full occlusion of the bacteria-containing tissues of the organ. Hatched areas represent diverticula of the ink sac, and stippled areas are the central portions of the ink sac complex that are covered with silvery tissue.

appear progressively larger, and cells in the transition between ciliated duct and nonciliated crypt epithelium appear massively enlarged (data not shown).

Microscopy of the lens and yellow filters

L-crystallin is the major protein component of the light organ's lens (Montgomery and McFall-Ngai, 1992), a structure that covers the entire ventrum of each lobe but thins near the center where the pore is situated (Figs. 5 and 6). The yellow filters, which are hemispherical, are situated between the lens and the bacteria-containing crypts and to either side of the pore—anterior and posterior (see Fig. 1). The filters, both in histological sections (Fig. 5) and in transmission electron micrographs (data not shown), appear to be composed largely of masses of fibrous cells. The inner core of each filter is formed from a branch of the ciliated duct. The epithelial cells of this inner core stained positively with an antibody raised to L-crystallin, although markedly less strongly than lens tissue, in which the majority of the soluble protein is L-crystallin. These data on the yellow filters indicate that the protein pools of these cells are enriched in either L-crystallin or a closely related (ALDH-like) protein that cross reacts with the L-crystallin polyclonal antibody (Fig. 6).

Late development of aposymbiotic animals

Five aposymbiotic animals, between 14 and 18 d post-hatch and averaging 2.0 mm in mantle length, were examined (Fig. 7). The light organs of all specimens were distinctly bilobed (Fig. 7C), and the ink sac diverticula resembled that of the wild-caught juvenile of 2.8 mm in mantle length (see Fig. 1B); *i.e.*, the lateral and medial diverticula had already begun to form. At this stage in development, the lens was not yet evident. Conspicuous on these aposymbiotic animals (Fig. 7D) were the vestiges of the superficial ciliated epithelial field of cells, which

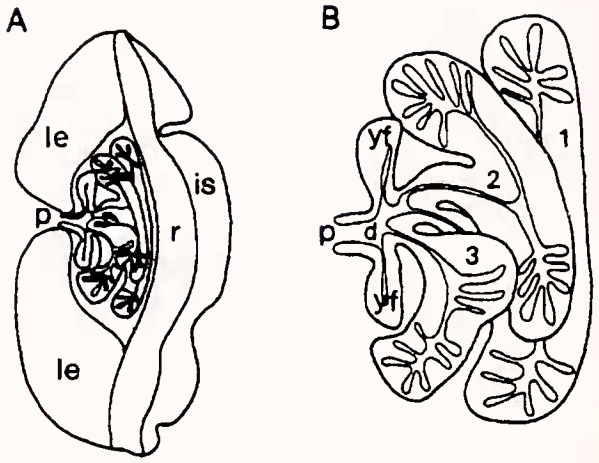

Figure 3. Relationship of the three bacteria-containing crypts (1, 2, and 3) to the ciliated duct (d) and the yellow filters (yf) in the left half of a fully differentiated light organ. (A) Frontal section through the left lobe of the light organ. (B) Enlargement of the crypts, yellow filters, and duct illustrated in (A). Diagrams based on examination of serial frontal sections of the light organ of a squid 8.0 mm in mantle length. is, ink sac; le, lens; p, pore; r, reflector. Bar, 250 μm.

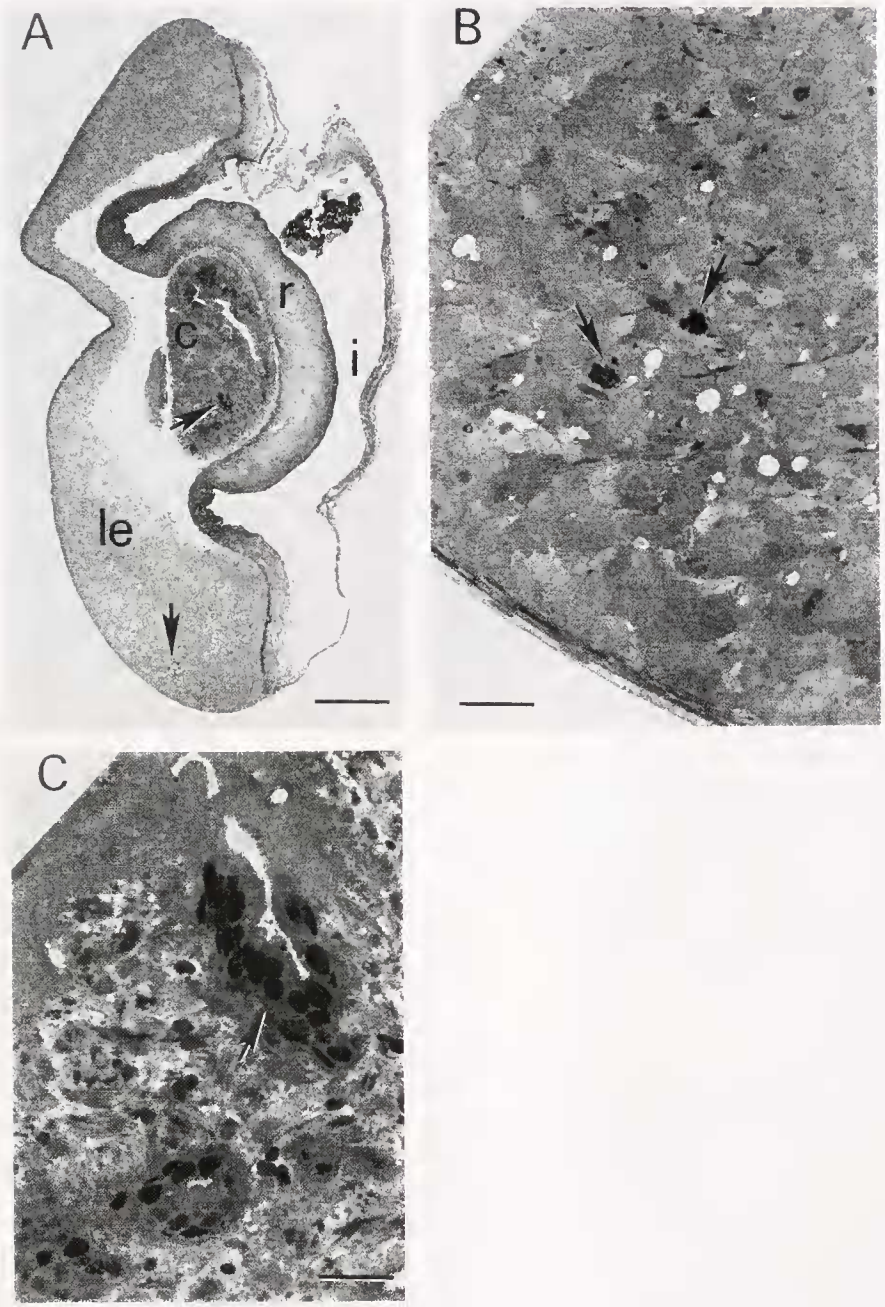

Figure 4. Incorporation of bromodeoxyuridine (BrdU) in a fully differentiated light organ. (A) Low-magnification micrograph of a frontal section through one lobe of a specimen 8.0 mm in mantle length exposed to 10 μM BrdU for 5 days. The section was labeled with anti-BrdU; note that the label is localized to cells in the most distal portions of the bacteria-containing crypts [arrow in crypts (c)] and in a few cells scattered throughout the lens [arrow in lens (le)]. The darkly pigmented area at upper right is a portion of the ink sac. Bar, 250 μm; r, reflector; i, ink sac. (B) The light organ lens at higher magnification; only a few nuclei are labeled in this tissue (arrows). Bar, 25 μm. (C) Labeled nuclei (arrow) in the distal portion of one of the branches of crypt 3. Bar, 25 μm.

in normal development and due to a program of bacteria-induced cell death, regresses within 4 d of hatching. Thus, although the change in shape and the elaboration of some accessory tissues occur in the absence of interaction with the symbionts, normal regression of the superficial ciliated field requires interaction with *V. fischeri*.

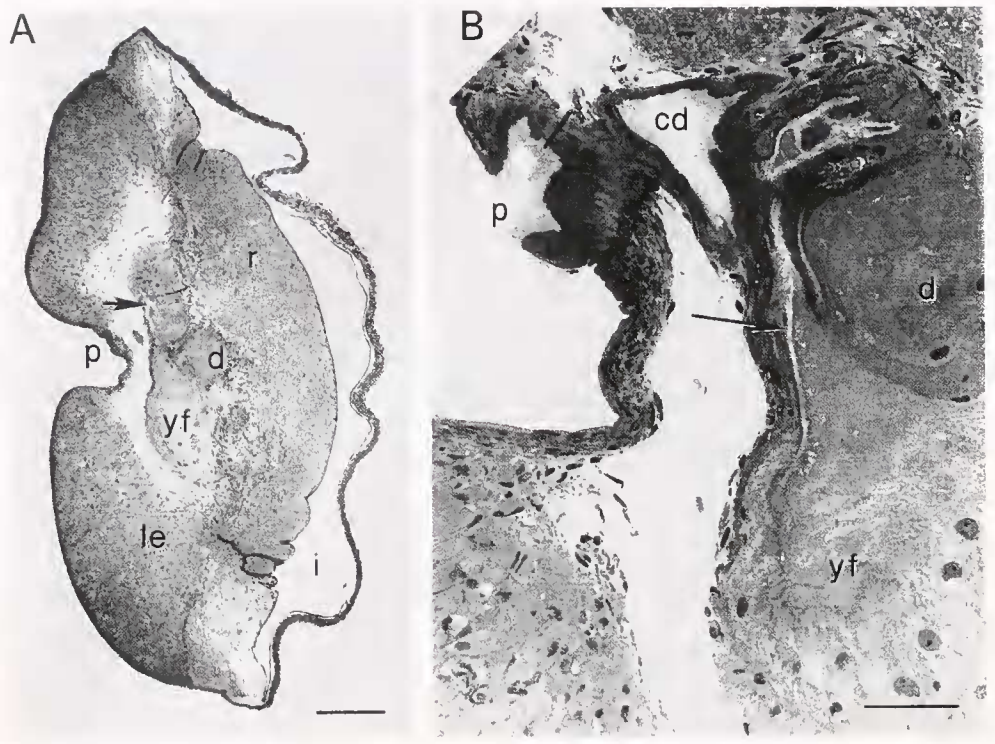

Figure 5. The yellow filters (yf) appear as a fibrous tissue outpocketing from the ciliated duct in these Richardson-stained frontal sections through one lobe of a mature light organ. (A) The posterior filter is shown fully in this section. The arrow indicates the lumen of the ciliated duct leading into the posterior filter. Bar, 250 μm. (B) This section reveals a portion of the ciliated duct (arrow) that leads from the pore region into the posterior filter (for orientation, see Fig. 3B). Bar, 50 μm. cd, ciliated duct; d, duct; le, lens; p, pore; r, reflector.

Discussion

The bacterial light organ of *E. scolopes* progresses through a series of developmental stages that reflect the changing function of the system. Specifically, a morphology associated with inoculation is replaced by a morphology that mediates the mature function of the symbiotic state, *i.e.*, the controlled emission of bacterial luminescence into the host's environment. The results of the present study, and reports from other investigators (Claes and Dunlap, 1998), show that the transition between these stages occurs within the first 2 weeks that follow the initial colonization with symbionts. The maturation program during this period involves significant remodeling of the organ, including (i) changes in shape, (ii) the loss, as well as the addition, of tissue types, and (iii) elaboration of already existing accessory structures (Table 1).

This work, combined with the our previous studies of the earlier developmental stages of this symbiosis (McFall-Ngai and Montgomery, 1990; Montgomery and McFall-Ngai, 1993, 1994), enable us now to compare the overall developmental sequence of the squid light organ with that of the well-described root-nodule symbiosis between leguminous plants and nitrogen-fixing bacteria, or rhizobia (for reviews see Hirsch, 1992; van Rhijn and Vanderleyden, 1995). The legume-rhizobia symbiosis has been studied for over 100 years, and until recently has been the only well-developed experimental system for the analysis of procaryotic-eucaryotic interactions; it has therefore been the system to which many others are compared and contrasted. In both the squid-vibrio and legume-rhizobia symbioses, within hours of the initial interactions of symbiosis-competent bacteria with host tissues (*i.e.*, either the crypt epithelium of hatchling squid light organs or the zone of susceptible root hairs on the legume), morphological changes are triggered both in the cells with which the bacteria directly associate and in remote tissues. Direct cell-cell interactions with *V. fischeri* trigger cell swelling (Montgomery and McFall-Ngai, 1994) and an increase in microvillar density in the crypt epithelium of the host squid (Lamarcq and McFall-Ngai, 1998), whereas rhizobia cause conspicuous root-hair curling. Remotely, an extensive program of apoptosis in the superficial field of host epithelia occurs in response to

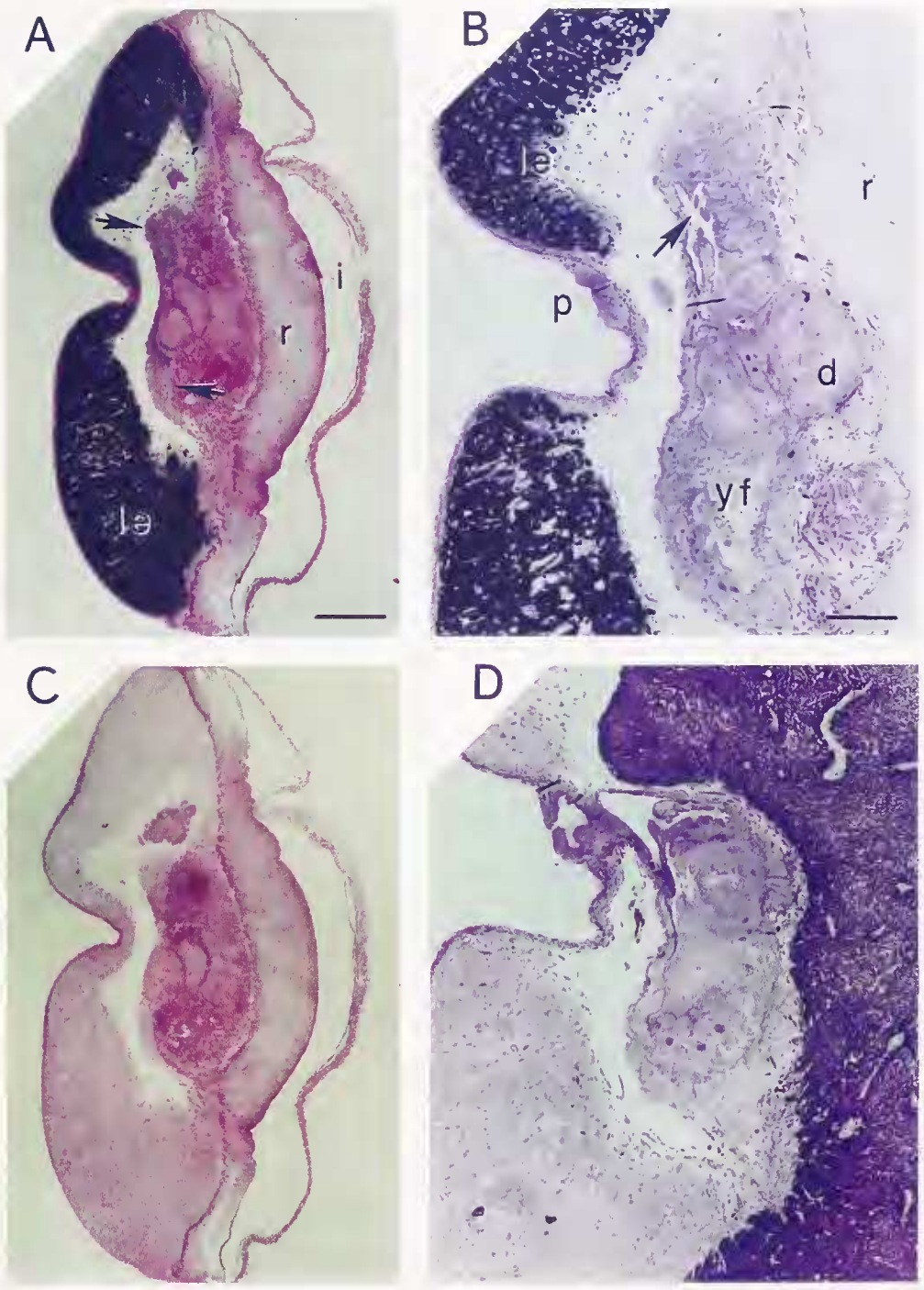

Figure 6. Immunocytochemistry of frontal sections through one lobe of the light organ of *Euprymna scolopes* (8.0 mm in mantle length) revealing production of a light organ lens protein, L-crystallin. (A) Low magnification of a section incubated with antiserum containing L-crystallin antibodies, counterstained with acid fuchsin. The silver granules indicate the high abundance of L-crystallin in the lens (le) (dense black), and the presence of the same or a closely related protein in the yellow filters (arrows), which are stained light gray. The ink in the ink sac has been washed out during fixation. i, ink sac; r, reflector; bar, 250 μm; anterior at top, ventral at left. (B) Higher magnification of the section in central region of the organ revealing the presence of ALDH-like protein in the posterior yellow filter (yf) and in the portions of the ciliated duct that lead into the yellow filter (arrow); low levels of the protein are also present in the pore (p) and in the portions of the duct (d) that leads to the bacteria-containing crypts. Bar, 100 μm. (C, D) No silver precipitate is seen in adjacent sections of the same light organ, at the same magnification, incubated with preimmune serum.

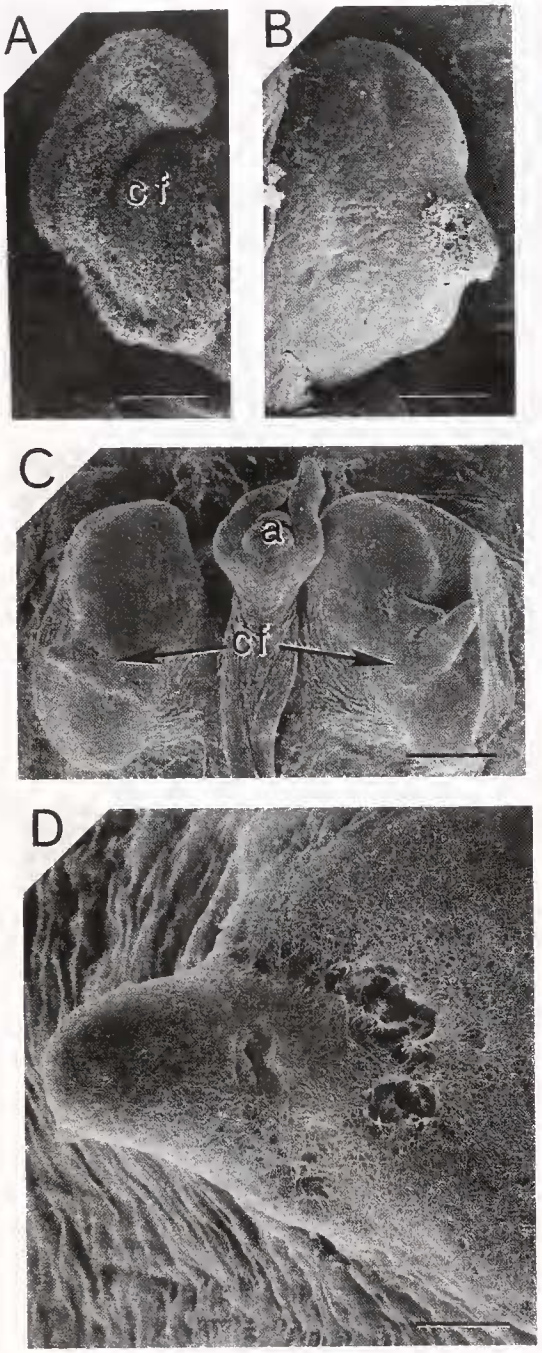

Figure 7. Scanning electron micrographs (ventral views) illustrating the developmental changes in the light organ that are independent of, or dependent upon, interactions with *Vibrio fischeri* (A) One half of the hatchling light organ showing the complex ciliated field (cf) on the lateral face of the ovoid organ. Bar, 50 μm. (B) One half of a 4-d-old symbiotic animal showing the nearly complete regression of the ciliated field. Bar, 50 μm. (C) Whole light organ of a 15-d-old aposymbiotic animal showing the bilobed shape of the organ and the retention of vestiges of the ciliated field (cf). a, anus; bar, 200 μm. (D) Higher magnification of the left side of (C) showing dense cilia on the lateral surface of the organ. Bar, 25 μm.

interactions with *V. fischeri* (Montgomery and McFall-Ngai, 1994; Foster and McFall-Ngai, 1998), whereas the signals from rhizobia induce cell division deep in the root cortex (Fisher and Long, 1992; Niner and Hirsch, 1998). However, clear differences in the later developmental patterns in these two types of symbiosis are also evident. Whereas bacterial bioluminescence—the principal contribution of the microbial symbiont—is induced in the squid organ within 8 to 10 hours after inoculation (McFall-Ngai and Ruby, 1991), nitrogen fixation by rhizobia does not occur at maximal levels in the root nodule for days to weeks. In addition, the squid light organ continues to grow throughout the life history of the host, and the bacterial symbionts continue to divide. In the legume-rhizobia symbioses, the active host cells of the root nodule have a finite lifetime, and the intracellular bacteria within the nodule form bacteroids, which represents a terminal differentiation of these cells (Hirsch, 1992).

A further critical difference between these symbioses is that the developmental program of the squid light organ is less reliant on bacterial signals. In the root nodule associations, reciprocal induction of gene expression by the partners is essential for all phases of nodule morphogenesis and maturation (Niner and Hirsch, 1998); *i.e.*, an already existing, functional root hair and root cortex are recruited and transformed into the nitrogen-fixing nodule as a direct result of interaction with rhizobia or their secreted gene products. In contrast, previous studies of the patterns of early development in the squid-vibrio relationship, and the results presented here on late development in this system, indicate that only some stages of development require interaction with *V. fischeri*. During embryogenesis, in the absence of bacteria, tissues are developed that are destined to have no other function than to serve as the future symbiotic light organ. Subsequently, the early posthatch morphogenetic changes, such as apoptotic loss of the ciliated field (Montgomery and McFall-Ngai, 1994) and change in microvillar density of symbiont-associated cells (Lamarcq and McFall-Ngai, 1998), have an absolute requirement for interactions with symbiotically competent *V. fischeri*. However, even in the absence of symbionts, the light organ of aposymbiotic animals converts from a rounded to a bilobed shape and elaborates the accessory structures of the organ. This result shows that these aspects of later development are genetically "hard wired"; that is, they do not depend on an inductive interaction with the symbionts.

The differences between the squid-vibrio and the legume-rhizobia symbioses in their requirements for bacterial induction during development may reflect a very basic difference in the nature of these associations. The squid-vibrio symbiosis appears to be obligate throughout the life history of the host; examination of several hundred

Table 1

Postembryonic development of the Euprymna scolopes *light organ*

Light organ feature	Newly hatched (≈1.6 mm ML)	Symbiotic (≥2.8 mm ML)	Development under *V. fischeri* induction*	Reference
Early development (hatching through day 4)				
ciliated surface epithelium	present	absent	yes	Montgomery and McFall-Ngai (1994)
microvilli of crypt epithelium	sparse	dense	yes	Lamarcq and McFall-Ngai (1998)
crypt epithelial cell shape	columnar	cuboidal	yes	Montgomery and McFall-Ngai (1994)
Late development (end of week 2 onward)				
light organ shape	ovoid	bilobed	no	This study
ink sac diverticula	anterior/posterior	anterior/posterior + paired medial/lateral	no	This study
lens	absent	present	no	Claes and Dunlap (1998)
crypts	sacculate	branching	no	Claes and Dunlap (1998)
yellow filters	absent	present	n.d.	This study

*n.d. = not determined.

field-caught animals of all ages has revealed that *E. scolopes* always has a functioning bacterial light organ (McFall-Ngai, pers. obs.). Because a symbiosis is always present in this species, we would expect portions of the developmental program to be independent of symbiont induction. In contrast, nodules form in legumes only when soil nitrogen is limiting (van Rhijn and Vanderleyden, 1995). Thus, the developmental program of the legume root has not incorporated nodule formation into its basic pattern, but only the genetic ability to respond and develop nodules under particular environmental conditions.

The coordinated late development of the accessory structures of the squid light organ, including the lens, reflector, and ink sac diverticula, reflects both the accommodation of these tissues to the changing size and shape of the organ and their function in the modulation of bacterial luminescence. However, the role of the yellow filters, which also arise later in development, is not as clear. Such filters are common in bioluminescence systems, and it has been suggested that they serve to shift the wavelength maximum of the emitted luminescence so that it more closely matches the spectral quality of environmental light (Denton *et al.*, 1985; McFall-Ngai, 1990). The notion that the yellow filters of the squid system function in this way is supported by some evidence. The wavelength maximum of *V. fischeri* luminescence is typically 489 nm (Eley *et al.*, 1970), and if the yellow filters of the *E. scolopes* light organ do shift the wavelength of bacterial light emission toward the red, then the luminescence emitted would more closely match the spectrum of downwelling moonlight, as well as the maximum absorption wavelength of the visual pigment of *E. scolopes*, which is 500–507 nm (F. Crescitelli, unpubl. data).

Although all recognized, late developmental events in the squid-vibrio association appear to be independent of symbiont induction, some of the bacteria-induced signaling of biochemical changes in the light organ that occur in early development are likely to require sustained interaction with the bacterial symbionts. For example, the requirement for continued interaction with bacterial symbionts to maintain the increase in microvillar density of the crypt epithelia has already been described (Lamarcq and McFall-Ngai, 1998). Taking advantage of the ability to eliminate the symbiont population from the light organ by treatment with antibiotics (Doino and McFall-Ngai, 1995), a comparison of the biochemistry of the light organ in cured and uncured adults should reveal the extent to which the composition of the internal environment of the host cells relies on the presence of the bacteria with which they directly interact.

The results obtained on late developmental patterns in the squid-vibrio association have demonstrated that the period during which bacteria induce developmental events is restricted to the first hours to days following inoculation. Studies of the bacterial symbiont have revealed that this is also the period during which *V. fischeri* displays dramatic genetic and morphological changes as it transforms from a free-living member of the bacterioplankton to a resident of the light organ (Ruby, 1996). Taken together, these findings indicate that the first hours to days of this symbiosis will continue to be the most fruitful period for discovering the biochemical and molec-

ular bases of the reciprocal dialogue that occurs between the partners of this symbiosis.

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